

*Interactive comment on “Decreased calcification affects photosynthetic responses of *Emiliana huxleyi* exposed to UV radiation and elevated temperature” by K. Xu, K. Gao, V. E. Villafañe, and E. W. Helbling*
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Reviewer: *General comments This manuscript reports on the coupling between calcification and photosynthesis in *Emiliana huxleyi* with respect to UV radiation and elevated temperatures. The authors showed that photosynthesis response to changes in UV radiation and temperature was dependent on the presence of coccoliths on the algae’s surface. This work provides new interesting data and although this is a purely physiological study, this subject remains central within the context of climate change and global warming, and the ongoing debate about the fate of calcifying organisms.*

Response: We appreciate the reviewer’s remarks and his/her comments that helped to improve our MS.

Reviewer: *Title: I think that it is more appropriate to change the title to “lack of coccolith on cell surface affects photosynthesis responses of *Emiliana huxleyi* exposed to UV radiation and elevated temperatures” because experiments have been conducted with coccolith-bearing and coccolith-less cells.*

Response: Calcification is an energy dependent process that is also dependent on photosynthesis, thus there is a strong feedback between the two processes. However, the reviewer has a point.

Action taken: We changed the title a bit to: Photosynthetic responses of *Emiliana huxleyi* to UV radiation and elevated temperature: Roles of calcified coccoliths

Reviewer: *I think that the experimental design used need much clarification because I could not reconsolidate the number of treatment and replicate done with the final number of tube used (36). I am obviously missing something. As I see it, for each calcium concentrations, you used 6 filters (280, 295, 305, 320, 350 and 395nm), and*

with 2 temperatures (20 and 25 °C), with 6 tubes for each (triplicates for measuring photosynthesis and calcification and triplicates for Pam measurements). That makes 72 tubes per calcium concentrations not 36. Plus, I am further confused concerning the PAR, UV-A and UV-B irradiance mentioned on p.861 l.8-10 and where they come into play in this experimental design.

Response: The reviewer is right in his/her analysis and perhaps this point was not clear in the original text. The only difference, and this is why we had a number of 36 tubes, is that the two temperatures were tested one after the other. As mentioned in the text, we used a solar simulator for a constant source of radiation, but one constraint is that the effective radiation area under it is limited and thus precluded us to test both temperatures at the same time. Since all the conditions were maintained the same, we did the experiments in two steps, one temperature at a time.

The reviewer is also right in that the mentioning of the three spectral ranges PAR (400-700 nm), UVA (315-400 nm) and UVB (280-315 nm) seems out of place in a “spectral experiment”. However, these are the spectral ranges defined by CIE (International Commission on Illumination) and we think that for comparative purposes with others that used these ranges it is important to give the irradiance levels at which the cells were exposed.

Action taken: We revised the manuscript according to reviewer’s comment to clarify the experimental design. We added a line that now reads: “Carbon incorporation and Pulse Amplitude Modulated (PAM) fluorescence measurements were done to assess the combined effects of ultraviolet radiation (UVR) and temperature on *Emiliana huxleyi*. Cells were exposed to artificial radiation under a solar simulator (Sol 1200W; Dr. Hönle, Martinsried, Germany) at a distance of 120 cm from the lamp for 2 h. The irradiances output were measured with a broadband ELDONET filter radiometer (Real Time Computers, Möhrendorf, Germany) that has channels for PAR (400-700 nm), UV-A (315-400 nm) and UV-B (280-315 nm), and the measured values were 63.5 W m^{-2} ($290 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), 23.1 W m^{-2} and 1.20 W m^{-2} , for PAR, UV-A and UV-B, respectively.

Biological weighting functions (BWFs) were used to separate the effects of

different UV wavebands on photosynthesis, calcification, effective photochemical quantum yield of PSII (Y) and non-photochemical quenching (NPQ). Six different radiation treatments were set by using Schott cut-off filters that cut radiation below 280, 295, 305, 320, 350 and 395 nm (the transmission spectra of these filters are published elsewhere, Villafañe et al., 2003). For each experimental Ca^{2+} concentration, the samples were dispensed in six quartz tubes (14 mL) per radiation treatment (i.e., under each Schott filter). Thus a total of 36 tubes were irradiated; half of them were used for measurements of photosynthetic carbon fixation and calcification whereas the other half was used for PAM measurements (see below). The tubes containing the samples were put in a water bath for temperature control at 20°C or 25°C using a circulating cooler (CTP-3000, Eyela, Tokyo, Japan). Due to the effective irradiance area under the solar simulator, it was not possible to test the effects of both temperatures at the same time, so experiments were done one after the other. In this way we were sure that the irradiance received by the cells were the same.”

Reviewer: P.864 l.24: “increase exposure time” - clarify, exposure time of what?

Response: The reviewer is right and the wording of this part was a bit difficult to follow. Since samples and measurements were done at 40, 80, and 120 min, the increased exposure time referred to these time spans of exposure under the solar simulator.

Action taken: We clarified the sentence that now reads: “Furthermore, at 20 °C, the Y differences among Ca^{2+} treatments decreased significantly ($p < 0.05$) as the experiment progressed (i.e., at different times of exposure to radiation).”

Reviewer: “P.865 l.11- 12: “HCa had lower inhibition than LCa treatment” but this does not look statistically different, in which case it is not different. P.865 l.10-19: I think that it is important to state in that paragraph that inhibition increase with exposure to UVR wavelengths.

Response: The reviewer has a point in this. As explained above the differences among HCa and LCa changed with exposure time, so there were significant differences at 40

and 80 min but not at 2 h. Perhaps this was not clear in the figure.

Action taken: We clarified the sentence that now reads: “In general, the *Y* inhibition increased with increasing exposure time to UV wavebands. HCa had significantly but slightly lower inhibition ($p < 0.05$) than LCa treatments at 40 min and 80 min, but there were no significant differences between the two Ca^{2+} concentrations after 2 h.”

Reviewer: P.867 l.14-16: point 2 needs to be rephrased to become clearer. For example using the term “performance” on its own is not precise enough. Performance in terms of what?

Response: The reviewer is right and we improved the text according to reviewer’s comment.

Action taken: We reworded point 2 that now reads: “(2) Calcification and photosynthesis (and their ratio) showed different responses related to UVR, with the HCa-grown cells having more tolerance to UVR than the LCa-grown ones;”

Reviewer: P.867 l.20: similarly, “also a negative...calcification” this statement need to be clarified. .

Response: We have improved the text according to the reviewer’s comment.

Action taken: We reworded the sentences and moved the “negative feedback” further down the text where we think it is now clearer.

Reviewer: 868 l.15-16. The authors should mention that this is a classical shade adaptation behavior.

Response: We agree with the reviewer.

Action taken: We reworded the sentence as suggested and now it reads: “Consequently, after long-term acclimation to less radiation the HCa-grown cells upregulated their contents of chl *a*, with an increase of the antenna size, reflecting a classical shade adaptation behavior.”

Reviewer: P.868 l. 18: again “performance” in terms of what

Response: We agree with the reviewer.

Action taken: The sentence was reworded and now it reads: “The *E. huxleyi* cells grown at the high Ca^{2+} concentration had higher photosynthetic carbon fixation, calcification rate and more tolerance to UVR than those grown at low Ca^{2+} concentration.”

Reviewer: P869 l.6-9: That’s true for cells grown and incubated at high [Ca] but for those grown and incubated at low [ca] calcification rates remained constant while NPQ decreased with increasing wavelength. The authors should discuss this point in terms of coccolith-bearing and coccolith-less cells.

Response: The reviewer is right and in fact, as stated in the original text, we were talking about the HCa grown cells, trying to understand the different possibilities that HCa cells has to cope with UVR. Nevertheless it is correct that coccolith-bearing cells (or HCa) were the ones with better “protection”

Action taken: We improved the text trying to accommodate the reviewer’s suggestion. These sentences were added in the Discussion section: “UVR can have a fast induction of NPQ by increasing PSII inactivation, reducing RUBISCO activity and enhancing xanthophylls de-epoxidation (Van de Poll et al., 2009). So, NPQ increased as shorter UV wavelengths were received by the cells (Fig. 4), but at the same time, photosynthetic carbon fixation and *Y* decreased (Figs. 2, 5).”

Reviewer: P.869 l.10-11: “reasonably....energy” I do not see what is the direct link between this sentence and the previous paragraph. The authors need to expend this point a bit more to clarify their thoughts.

Response: We agree in that our wording was not clear.

Action taken: We reworded this part to accommodate the reviewer’s comment. The sentence now reads: “It is known that as an energy dependent process, calcification involves the cellular uptake of dissolved inorganic carbon and Ca^{2+} from the extracellular medium into the coccolith vesicle and the production of coccolith polysaccharides (Brownlee and Taylor, 2004).”

Reviewer: P.871 l.15-25: *I am surprised of the lack of mention of the Iglesias-Rodriguez et al 2009 Science paper, which is important within the context of E. huxleyi response to ocean acidification*

Response: The reviewer is right and we added the reference of Iglesias-Rodriguez et al 2009 as suggested.

Action taken: This sentence was added in the Discussion section: “Many studies focused on the effects of ocean acidification on coccolithophore (Riebesell et al., 2000; Iglesias-Rodriguez et al., 2008; Feng et al., 2008; De Bodt et al., 2010), but few considered the role of UVR in natural conditions (Gao et al., 2009).”

Reviewer: p.858 l.20: *add “producing” between “as well as” and “calcium”*

p.859 l.25: *remove “and” before “considering a context”*

p.864 l.22: *remove “and” before “at 20”*

p.867 l.16: *after “as compared to” remove one of the first “the”*

Fig 2 panel C: use “Y25/20” for the right vertical axis, which would then agree with what you used in the text.

For all figures that report wavelength in horizontal axis, add PAR, UVB, UVA and UVR in addition to the actual wavelength on the horizontal axis, because this is how the data is discussed in the main text and it would make life easier to match text and figure.

Fig. 2 legend: exposure to what? Clarify

Fig 7 panel C: add “BWf of “ before “fixation”

Response: We agree with these points made by the reviewer and we changed all of them as suggested.